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## Functioning of a metabolic flux sensor in *Escherichia coli*

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*Published in:*

Proceedings of the National Academy of Sciences of the United States of America

*DOI:*

[10.1073/pnas.1202582110](https://doi.org/10.1073/pnas.1202582110)

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2013

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Kochanowski, K., Volkmer, B., Gerosa, L., Haverkorn van Rijsewijk, B. R., Schmidt, A., & Heinemann, M. (2013). Functioning of a metabolic flux sensor in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 110(3), 1130-1135. <https://doi.org/10.1073/pnas.1202582110>

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# Supporting Information

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## SI Text 1: Model Describing System Without Feedforward Activation

The mathematical model containing two metabolites ( $X$  and  $Y$ ) and two enzymatic reactions ( $E_1$  and  $E_2$ ) can be described by a set of two ordinary differential equations:

$$\frac{dX}{dt} = v - v_{E1} \quad [\text{S1}]$$

$$\frac{dY}{dt} = v_{E1} - v_{E2}, \quad [\text{S2}]$$

where  $v$  denotes the influx (input of the system) and  $v_{E1}$  and  $v_{E2}$  denote the reaction rates of  $E_1$  and  $E_2$ , respectively. At steady state, the differential expressions are zero, simplifying Eqs. S1 and S2 to the following:

$$v_{E1} = v_{E2} = v. \quad [\text{S3}]$$

The rates through the two reactions  $v_{E1}$  and  $v_{E2}$  can be described with the following equations, assuming a reversible Michaelis-Menten-type kinetic for  $E_1$  and an irreversible Michaelis-Menten-type kinetic for  $E_2$ :

$$v_{E1} = v = \frac{v_{\max,E1} \cdot \left( X - \frac{Y}{K_{eq}} \right)}{K_{m,X,E1} \cdot \left( 1 + \frac{Y}{K_{m,Y,E1}} \right) + X} \quad [\text{S4}]$$

$$v_{E2} = v = \frac{v_{\max,E2} \cdot Y}{K_{m,Y,E2} + Y}. \quad [\text{S5}]$$

$K_{m,X,E1}$ ,  $K_{m,Y,E1}$ , and  $K_{m,Y,E2}$  denote the  $K_m$  values for  $X$  and  $Y$  of  $E_1$  and for  $Y$  of  $E_2$ , respectively.  $K_{eq}$  denotes the equilibrium constant of  $E_1$ , and  $v_{\max,E1}$  and  $v_{\max,E2}$  denote the maximal possible fluxes of  $E_1$  and  $E_2$ , respectively.

To obtain an analytical solution of the relationship of  $X$  and the flux  $v$ , Eqs. S4 and S5 can be rearranged for  $X$  and  $Y$ , respectively. Replacing  $Y$  in Eq. S4 (and assuming that  $K_{m,X,E1} =$

$K_{m,Y,E1}$  to reduce the number of parameters), one obtains the following:

$$X = \frac{v \cdot K_{m,X,E1}}{v_{\max,E1} - v} + \frac{v \cdot K_{m,Y,E2} \cdot v_{\max,E1}}{K_{eq} \cdot (v_{\max,E1} - v) \cdot (v_{\max,E2} - v)} + \frac{v^2 \cdot K_{m,Y,E2}}{(v_{\max,E1} - v) \cdot (v_{\max,E2} - v)}. \quad [\text{S6}]$$

For  $v \ll v_{\max,E1}$  and  $v \ll v_{\max,E2}$  (which is equivalent to an influx that is much lower than the maximal possible fluxes for  $E_1$  and  $E_2$ ), this equation simplifies to the following:

$$X = \frac{v \cdot K_{m,X,E1}}{v_{\max,E1}} + \frac{v \cdot K_{m,Y,E2}}{K_{eq} \cdot v_{\max,E2}}. \quad [\text{S7}]$$

This equation describes the analytical solution for the relationship of  $X$  and flux  $v$ .

## SI Text 2: Model Describing System with Feedforward Activation of $E_2$ by $X$

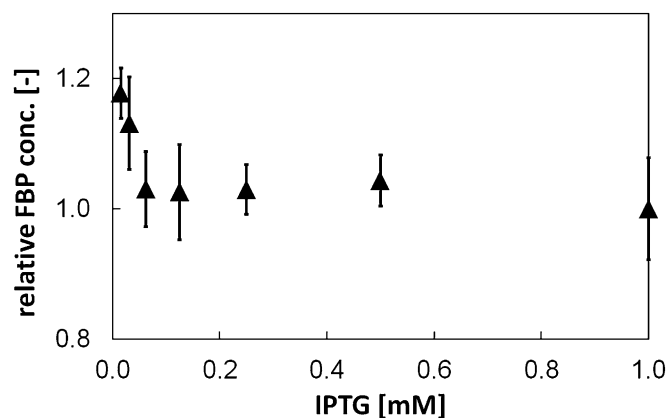
Here, we use the same ordinary differential equations as above. In contrast to the previous model, we use a Monod-Wyman-Changeux (MWC) kinetic for  $E_2$  in accordance to previous studies on pyruvate kinase I (PYK I) (1–3):

$$v_{E2} = v = \frac{v_{\max,E2} \cdot \frac{Y}{K_{m,Y,E2}} \cdot \left( 1 + \frac{Y}{K_{m,Y,E2}} \right)^{n-1}}{L \cdot \left( 1 + \frac{X}{K_{mA,X,E2}} \right)^{-n} + \left( 1 + \frac{Y}{K_{m,Y,E2}} \right)^n}. \quad [\text{S8}]$$

$L$ ,  $n$ , and  $K_{mA,X,E2}$  in Eq. S8 denote allosteric equilibrium constant, cooperativity constant, and affinity constant of  $X$  for  $E_2$ , respectively. We chose values for  $L$  and  $n$  in accordance to parameter values that were obtained for PYK I in previous studies: PYK I is inactive in absence of its allosteric activator FBP (1, 4), which corresponds to  $L \gg 1$ , and several studies have determined  $n$  to be equal to 4 (1, 5). Because  $n > 1$ , it is not possible to derive an analytical solution for the relationship of  $X$  and flux  $v$ , and thus we solve this model equation as specified in the main text.

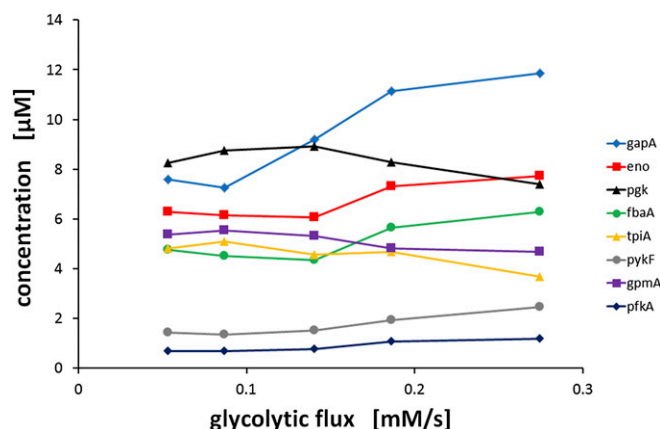
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**Fig. S1.** Abundance of intracellular FBP as a function of IPTG concentration (used as a proxy for *pykF* abundance) in glucose batch cultures of a *pykF* mutant strain bearing an IPTG-inducible PYK I expression plasmid, relative to the FBP concentration at 1 mM IPTG. Batch cultivations were performed in 500-mL shake flasks containing 30 mL of M9 glucose medium (5 g/L glucose) as described in the main text. Cultures were harvested in midexponential growth phase using fast filtration and extracted in hot ethanol as described previously (1). Extracts were then analyzed by LC-MS/MS as described in the main text.

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**Fig. S2.** Intracellular concentrations of enzymes of lower glycolysis at different glycolytic fluxes. Whereas the data from the four lower flux values stem from glucose-limited chemostat cultures, the highest flux data point was obtained from a glucose batch culture. Enzyme copy numbers were determined by targeted MS analysis and heavy reference peptides (1) and then converted to concentrations using the cell volumes measured in ref. 2.

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